

Applicant : Jacob Bar-Tana
Serial No.: 10/735,439
Filed : December 11, 2003
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In the specification:

Please replace the paragraph beginning on page 10, line 12, with the following amended paragraph:

Long chain fatty acids are shown here to directly modulated the transcriptional activity of ~~HNF-4~~, preferably HNF-4 α [[,]] by binding of the respective fatty acyl-CoA thioesters to the HNF-4 α ligand binding domain. Transcriptional modulation by HNF-4 α agonistic or antagonistic acyl-CoA ligands may result from two apparently independent ligand induce effects, namely, shifting the HNF-4 α oligomeric-dimeric equilibrium or affecting the intrinsic binding affinity of the HNF-4 α dimer for its cognate enhancer.

Please replace the paragraph beginning on page 12, line 17, and ending at page 13, line 14, with the following amended paragraph:

Reaction mixture contained 20 mM Hepes-KOH (pH 7.9), 5mM MgCl₂, 60 mM KCl, 8% glycerol, 2 mM DTT, 1 mM 3'-O-methyl-GTP, 10 units of T1 RNase, 20 units of RNasin, 0.5 μ g sonicated salmon sperm DNA and His-HNF-4 α and test ligand as indicated. The mixture was preincubated for 30 min at 22°C followed by adding 10 ng of pAdML200 control template consisting of the adenovirus major late promoter (-400/+10) linked to a 200 bp G-less cassette and 200 ng of the test template consisting of three C3P copies of the apo CIII promoter sequence (-87/-66) upstream to a synthetic ovalbumin TATA box promoter in front of a 377

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bp-G-less cassette. The mixture was further preincubated for 10 min at 22°C followed by adding 40 μ g of HeLa nuclear extract with additional preincubation for 30 min at 30°C. 0.5 mM ATP, 0.5 mM CTP, 25 μ M UTP, and 10 μ Ci of [α -³²P]UTP (s.a. 800 Ci/mol, Amersham) were then added and the complete reaction mixture was incubated for 45 min at 30°C in a final volume of 25 μ l. The reaction was terminated by adding 175 μ l of stop mix (0.1 M sodium acetate (pH 5.2), 10 mM EDTA, 0.1% SDS, 200 μ l/ml tRNA) followed by phenol extraction and ethanol precipitation. RNA was resuspended in sample buffer containing 80% formamide and 10 mM Tris-HCL (pH 7.4) and separated on 5% polyacrylamide gel containing 7 M urea in TBE. Correctly initiated transcripts were quantitated by PhosphorImager analysis. The test DNA template was constructed by inserting into pC₂AT19 plasmid a PCR-amplified oligonucleotide prepared by using the (C3P)₃-TK-CAT plasmid as template and consisting of three copies of the C3P element of the Apo CIII promoter sequence (-87/-66) having an EcoRI and SSTI sites at the 5' and 3' ends, respectively. The resultant plasmid was cleaved with sphI and sacI and ligated to a synthetic oligonucleotide (5'-CGAGGTCCACTTCGCTATATATTTCCCGAGCT-3') (SEQ ID NO:1) containing sequences of the HSV thymidine kinase promoter (-41/-29) and of the chicken ovalbumin promoter (-33/-21).